about vulnerable individuals like the elderly, children, or asthmatics. After exposure over a long time to this type of pollution, these individuals experience even more difficulty surviving a health crisis like a heart attack or pneumonia."

The study tracked 8111 men and women over a period of 14–16 years for risk factors such as smoking status, obesity, occupational risks, and low education. The six cities in the study are Watertown, Massachusetts; Harriman, Tennessee, including Kingston; certain census tracts in St. Louis, Missouri; Steubenville, Ohio; Portage, Wisconsin; and Topeka, Kansas. The type of air pollution described in the study typically contains a mix of soot, acid condensates, sulfates, and nitrate particles. Under current EPA standards, cities are considered out of compliance if the 24-hour average concentration of particles 10 microns or smaller (PM_{10}) exceeds 150 micrograms per cubic meter ($\mu g/m^3$) of air or the annual mean exceeds 50 $\mu g/m^3$. In contrast, the particles most strongly associated with mortality in the study were 2.5 microns or smaller in diameter.

Fine particles come from auto exhausts, factory and power plant smokestacks, and other processes that burn coal, oil, and natural gas. Such particles contain sulfur, carbon (soot), various metals, and droplets of sulfuric acid. The white haze often seen on hot, humid days is evidence of this type of pollution. Usually larger solids such as dirt, dust, ash, and pollen are trapped in the nose, mouth, and windpipe and are removed by coughing, nose blowing, and expectoration. Farther down the respiratory tract, a mucous layer traps smaller flecks of particles and transports them upward in phlegm. Inevitably, however, tiny particles work their way into the alveoli, the terminal site of air exchange in the lung. Sulfur, metals, soot, and acid in the alveoli may irreversibly damage these sensitive tissues, permanently reducing lung capacity. Consequently, individuals exposed to this type of pollution, and impaired by other diseases or weakened by age, are very vulnerable.

Other studies corroborate the same link between death and pollution at levels less than the federal standard. These include a study in Philadelphia showing that deaths start to increase when particles reach levels of one-third the current legal standard.

"No one study can prove causality," Dockery cautions. "Statistical analyses like this one don't reveal the causes of sickness and death, but they point out strong associations. We now have a good case for the inadequacy of the EPA standards now in effect. All this information points to the need to reduce the amounts of airborne particles to which the general public and more vulnerable people, such as those with lung and heart problems, are exposed," he concluded.

Milwaukee MFBS Center

Dioxin injection. Graduate student Patrick Guiney and Barbara Wimpee inject lake trout eggs with TCDD.

The Six-Cities Study began in 1974 as a prospective epidemiological investigation of the health effects of ambient sulfur dioxide and particulate matter on respiratory health of children and adults in six selected U.S. cities. In 1987, a companion study was initiated to include 24 cities in the United States and Canada to assess the adverse respiratory health effects of acid air pollutants on preadolescent children. Data from these studies have been used extensively as the basis for setting clean air standards in the United States and Canada.

Alternative Assays

The National Institutes of Health Revitalization Act, signed by President Clinton in June 1993, directs the NIEHS to develop and validate new assays and protocols for safety testing, including alternative methods to standard mammalian laboratory animals such as rodents. While the biomedical science community will require the use of animal models for the foreseeable future, current goals include applying the "three R's": reduction of animal use, refinements to enhance the well-being of animals, and replacement of animal models where possible. The NIEHS mandate includes establishing specific criteria for scientifically validated alternative methods, thus increasing the likelihood that these methods will be acceped by regulatory agencies.

NIEHS Director Kenneth Olden was a featured speaker at the World Congress on Alternatives and Animal Use in the Life Sciences in November, which NIEHS cosponsored. Olden stated that NIEHS spends $21 million a year on the development of new assays, about 1/12 of its budget. "Since this is the basis of our research," Olden said, "I think we should be spending more."

He noted that promising alternative assays include nonmammalian species such as the frog embryo, small fish, sea urchin, and *Drosophila* (fruitfly). Olden cited NIEHS Marine and Freshwater Biomedical Science Centers funded at five universities around the United States as conducting excellent research to develop aquatic species as laboratory animals and sentinel species.

Benefits of alternative methods include decreasing the time needed to complete an assay and lowering the cost. The standard rodent study takes two years in the experimental phase, plus time for study design and data analysis, and costs more than $2 million. It is estimated that there are about 62,000 synthetic chemicals in use in the United States, many thousands of which need screening. Olden also pointed out that cancer is not the only disease endpoint of concern; reproductive and developmental toxicity, neurotoxicity, immunotoxicity, and pulmonary toxicity are among the areas where rapid, relatively inexpensive assays are needed.

NIEHS has prepared a fact sheet on alternative assays: "NIEHS and the Use of Alternative Methods in Toxicological Research and Testing," available from the Office of Communications, NIEHS, MD WC-03, PO Box 12233, Research Triangle Park, NC 27709.